



DRA - CPC



2.D.2. Disk Retention Assay Performance Test for Cetylpyridinium Chloride Rinses (Appendix 2)

In evaluating CPC availability and biological activity in mouthwash formulations, Procter & Gamble recommends the use of the *in vitro* Disk Retention Assay (DRA) to estimate CPC drug availability, as well as the *ex vivo* Plaque Glycolysis and Regrowth Model (PGRM) to assess biological activity.

2.D.2.1. Scientific Basis of the DRA Test

In the August 15, 1995 review of the CPC clinical data, the Plaque Subcommittee observed that Procter & Gamble's 0.05% CPC mouthwash reduced both plaque and gingivitis while a Marion Merrell-Dow commercially marketed rinse (Cepacol®) containing the same formulated level of CPC only reduced plaque. Considering the potential for mouthwash excipients to reduce the antimicrobial efficacy of quaternary ammonium compounds, differences in CPC compatibility may have contributed to the clinical discrepancies observed between the two mouthwashes.

Cetylpyridinium chloride (CPC) is a quaternary ammonium compound with an aliphatic chain (C=16) and is classified as a cationic surface-active agent³². As such, it has both a positively charged hydrophilic region and a hydrophobic region. CPC has been shown to possess antimicrobial activity against a number of oral bacteria³³. The mechanism of action of CPC is dependent upon the ability of this positively charged molecule to interact with negatively charged anionic sites on the cell walls of bacteria.

³² The United States Pharmacopeia--23, The National Formulary—18, United States Pharmacopeial Convention, Inc., Rockville, Maryland, p. 329, 1995.

³³ Kolenbrander E., "Inhibition of Intergeneric Coaggregation Among Oral Bacteria by Cetylpyridinium Chloride, Chlorhexidine Digluconate and Octenidine Dihydrochloride. " J of Periodontal Research, 26: 422-428. 1191.

Under physiological conditions, bacterial cells carry a net negative charge as a result of the presence of negatively charged molecules on the cell surface. When bacteria are exposed to CPC, the positively charged hydrophilic group associates with the negatively charged groups on the bacterial surface allowing the hydrophobic portion of CPC to interact with the cell membrane resulting in leakage of cellular components, disruption of bacterial metabolism, inhibition of cell growth, and cell death^{34,35,36}.

Critical to CPC's antimicrobial activity is the availability of its positively charged hydrophilic region to facilitate attachment to bacterial and mucosal surfaces. Attachment to bacterial surfaces is necessary to achieve cell lysis during CPC exposure while binding to mucosal surfaces helps to establish a CPC reservoir during treatment. Common excipients added to commercial oral care formulations can diminish or even completely neutralize the antimicrobial activity of CPC³⁷ if not formulated correctly. The degree to which CPC's activity is decreased is determined by the choice and concentration of excipient added. Therefore, it is essential to establish that the CPC in mouthrinse formulations is sufficiently available and biologically active to result in an antigingivitis benefit.

In evaluating CPC availability and biological activity in mouthwash formulations, Procter & Gamble utilizes the *in vitro* Disk Retention Assay (DRA) to estimate CPC drug availability and the *ex vivo* Plaque Glycolysis and Regrowth Model (PGRM) to assess biological activity. Our experience has been that results from these models are

³⁴ Scheie, A. A., Modes of Action of Currently Known Chemical Antiplaque Agents Other Than Chlorhexidine, J. Dent. Res. 68:1606-1616, 1989.

³⁵ Smith, R.N. et al. Inhibition of Intergeneric Coaggregation Among Oral Bacteria by Cetylpyridinium Chloride, Chlorhexidine Digluconate and Octenidine Dihydrochloride, J. Period. Res. 26:422-429, 1991.

³⁶ Merianos, J.J. Quaternary Ammonium Antimicrobial Compounds. In Disinfection, Sterilization and Preservation, edited by S.S. Block, 4th edition, Lea & Febiger Co., Philadelphia, PA, pp. 225-255, 1991.

³⁷ OTC Volume 210013

reproducible, precise and accurate and reflect the availability and biological activity of CPC. Procter & Gamble relies on the empirical observations that within the appropriate class of antimicrobial agents, DRA and PGRM testing broadly correlates to clinical outcomes. It has been P&G's experience that DRA testing is especially sensitive to detecting changes in the availability of quaternary ammonium compounds (QUAT), such as CPC, and that increased levels of available QUAT correspond to increased biological activity and clinical efficacy. For cationic antimicrobial agents, the correlation between PGRM testing and clinical results is also relatively straightforward. For example, PGRM testing predicts that stannous fluoride, chlorhexidine and CPC containing oral care formulations would possess antigingivitis activity and in fact all of these agents have been shown to be clinically effective when properly formulated.^{38,39,40}

2.D.2.2. DRA Performance Test Method

The Disk Retention Assay (DRA) was previously reported to the Subcommittee during its deliberations. This method is designed as a performance assay to analyze rinse formulations containing 0.03% to 0.1% cetylpyridinium chloride (CPC) to quantitatively determine the free or "available" level of CPC.

The DRA assay test measures the amount of CPC "binding" to standardized cellulose filter disks during filtration of a undiluted mouthrinse sample providing an estimate of the availability of CPC from a rinse formulation. The "available" CPC is bound to hydroxyl groups on the cellulose fiber during filtration while CPC which has been rendered "unavailable (bound)" through interactions with mouthwash components

³⁸ Mandel, I.D. et al.: Chemotherapeutic Agents for Controlling Plaque and Gingivitis. *J. Clin. Periodontol.* 15:488-498, 1988.

³⁹ Perlich, M.A., et al.: The Clinical Effect of a Stabilized Stannous Fluoride Dentifrice on Plaque Formation, Gingivitis, and Gingival Bleeding: A Six Month Study. *J. Clin. Dent.* 6 (Special Issue): 54-58, 1995

⁴⁰ Ciancio, S.G. et al.: Agents for the Management of Plaque and Gingivitis. *J. Dent. Res.* 71: 1450-1454, 1992.

simply passes through the filter paper as the positive charge on the compound is no longer available for binding to the cellulose. In this way, the DRA test provides an estimate of the amount of CPC available for binding to bacteria and mucosal surfaces during treatment. In our laboratories, DRA measurements of CPC availability have been positively correlated to the results of *in vitro* microbiological assays and *in vivo* germ kill tests. Historically, cellulose fibers have been used in other applications to monitor biological activity. For example, these materials are accepted by the FDA and WHO as antibiotic control disk standards as well as by the dairy industry as a standard method for monitoring penicillin levels in milk.^{41,42}

“Available” CPC is the amount of CPC adsorbed to cellulose disks. This is determined by measuring the difference in CPC concentration in the mouthrinse before and after exposure to the cellulose disks. The method has been validated and shown to perform acceptably with accuracy, precision, and selectivity. A summary of the method and the analytical figures of merit are provided in Appendix 2.

2.D.2.3. Effectiveness Criteria for DRA Performance Test

Any 0.045 to 0.1% CPC-containing mouthrinse formulation passes the DRA test if results show its level of bioavailable CPC is $\geq 360\text{ppm}$ [$0.05\% \text{ CPC} \times 72\% \text{ bioavailability} = 360\text{ppm CPC}$]. The CPC products used in the clinical studies presented to the Subcommittee were all characterized as exhibiting between 72% and 77% bioactive CPC in the mouthrinse product. As such, the Subcommittee recommended that oral rinse products formulated with CPC maintain between 72% and 77% activity as determined via the DRA methodology. Determination of CPC bioavailability in a finished product is important to product performance as it readily defines the amount (concentration) of drug available for deposition at the site of action.

⁴¹ Dairy Products: in: *Official Methods of Analysis of the Association of Chemical Analytical Chemists*. 13th ed., Washington, D.C., Association of Official Analytical Chemists, Chapter 16:256, 1980.

⁴² Reference omitted

This characterization was acknowledged by the Subcommittee and determined to be an important parameter for characterizing CPC rinse products:

“Because the positively charged hydrophilic region is critical to antimicrobial activity, any formulation that diminishes the activity of this cationic group or that competes with the group may inactivate the product. Therefore, it is essential to establish that the cetylpyridinium chloride in products is sufficiently biologically active to justify an antigingivitis claim.”⁴³

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“Thus, it is clear that inactivation of cetylpyridinium chloride is likely based upon formulation. It is recommended that the bioavailability of cetylpyridinium chloride in each formulation be determined to reduce the possibility that the active ingredient is removed (rendered unavailable) due to chemical reaction, complexing, micelle (a colloid particle formed by an aggregation of small molecules) formation, or other sources of deactivation.”⁴⁴

While Procter & Gamble has not determined the absolute minimum threshold of bioactive CPC which defines the threshold between clinical success and failure, we have substantiated that 0.05% CPC mouthrinses yielding DRA results equal to or greater than 360 ppm CPC availability are clinically effective for the reduction of plaque and gingivitis. It is our recommendation that the DRA success criteria not be constrained to a narrow acceptable range (72% – 77%) of CPC availability based on formulated amount, but rather, that DRA effectiveness criteria be established as a

⁴³ Federal Register: 68 (32247), May 29, 2003

⁴⁴ Federal Register: 68 (32248), May 29, 2003

pass/fail criteria based on clinically relevant CPC threshold of ≥ 360 ppm availability. As demonstrated in the CPC clinical trials discussed during the Subcommittee deliberations and as subsequently recommended by the Subcommittee, the proposed monograph concentration for CPC is recommended to be between 0.045% to 0.1% CPC. Based on this recommendation and the performance criteria characterizing the lowest recommended concentration of an oral rinse product would be 324ppm CPC ($0.045\% \times 0.72$). It is appropriate to use the recommended DRA performance testing to predict (ensure) clinically relevant therapeutic outcomes. Therefore, P&G recommends that effectiveness criteria for DRA performance testing consist of the test product meeting or exceeding the DRA testing results of the recognized CPC performance standard. Further, P&G recommends that the minimal acceptable criteria for DRA be based on the concentration of a CPC oral rinse containing 0.05% CPC of which 72% of the CPC is available. This translates to a quantitative pass/fail value of 360ppm CPC ($0.05\% \times 0.72$). It is also recommended that the performance standard (positive control) for DRA be recognized as an analytically prepared aqueous solution containing 360ppm of available CPC.

2.D.2.4. Reference Standards for DRA Testing

Utilization of an analytical prepared reference standard (positive control) containing USP CPC appropriately mixed in deionized water to yield a 0.036% CPC solution would eliminate the possibility of CPC deactivation due to excipients and would be readily available to all manufacturers for DRA testing. Further, this reference standard would define a pass/fail threshold for all formulated (0.045 – 0.1%) concentrations of CPC. Procter and Gamble is willing to work with US Pharmacopoeia representatives to establish and supply both CPC and SnF_2 reference standards needed for the OTC Antigingivitis/Antiplaque Monograph.